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(54) Title: RAPAMYCIN DIALDEHYDES

(57) Abstract: This invention provides non-immunosuppressive rapamycin dialdehydes, which are useful as neurotrophic agents. in the treatment of solid tumors, and vascular disease.

RAPAMYCIN DIALDEHYDES

This invention relates to non-immunosuppressive rapamycin dialdehydes useful as neurotrophic agents, in the treatment of solid tumors, and hyperproliferative vascular disease; to processes for their preparation and to pharmaceutical compositions containing them.

Rapamycin is a macrocyclic triene antibiotic produced by Streptomyces hygroscopicus, which was found to have antifungal activity, particularly against Candida albicans, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S.N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Patent 3,929,992; and U.S. Patent 3,993,749]. Additionally, rapamycin alone (U.S. Patent 4,885,171) or in combination with picibanil (U.S. Patent 4,401,653) has been shown to have antitumor activity.

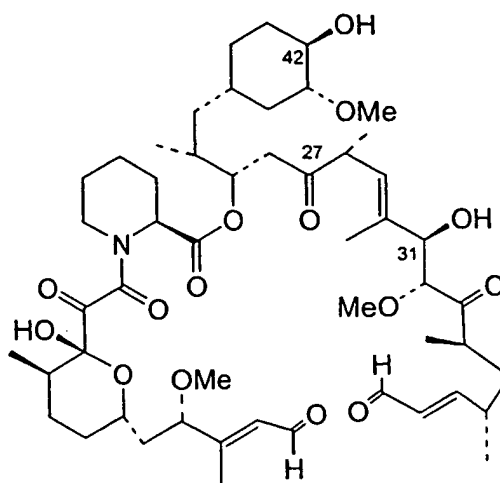
The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); R. Y. Calne et al., Lancet 1183 (1978); and U.S. Patent 5,100,899]. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies. FK-506 and some synthetic FKBP-12 binding ligands have been shown to be neuroprotective and neuroregenerative [US5696135, US5721256, US5780484, US5811434 and US 5840736].

Rapamycin is also useful in preventing or treating systemic lupus erythematosus [U.S. Patent 5,078,999], pulmonary inflammation [U.S. Patent 5,080,899], insulin dependent diabetes mellitus [U.S. Patent 5,321,009], skin disorders, such as psoriasis [U.S. Patent 5,286,730], bowel disorders [U.S. Patent 5,286,731], smooth muscle cell proliferation and intimal thickening following vascular injury [U.S. Patents 5,288,711 and 5,516,781], adult T-cell leukemia/lymphoma [European Patent Publication 525,960 A1], ocular inflammation [U.S. Patent 5,387,589], malignant carcinomas [U.S. Patent 5,206,018], cardiac inflammatory disease [U.S. Patent 5,496,832], anemia [U.S. Patent 5,561,138] and increase neurite outgrowth [Parker, E. M. et al, Neuropharmacology 39, 1913-1919, 2000].

A rapamycin ester, rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid [disclosed in U.S. Patent 5,362,718], also known as CCI-779, has been shown to have antitumor activity against a variety of tumor cell lines, in *in vivo* animal tumor models, and in Phase I clinical trials. [Gibbons, J., Proc. Am. Assoc. Can. Res. 40: 301 (1999); Geoerger, B., Proc. Am. Assoc. Can. Res. 40: 603 (1999); Alexandre, J., Proc. Am. Assoc. Can. Res. 40: 613 (1999); and Alexandre, J., Clin. Cancer. Res. 5 (November Supp.): Abstr. 7 (1999)].

DESCRIPTION OF THE INVENTION

10 This invention provides non-immunosuppressive rapamycin dialdehydes, and methods of using them that are described herein. As defined herein, the term "a rapamycin dialdehyde" defines a class of neurotrophic compounds which contain the basic rapamycin dialdehyde nucleus (shown below). These compounds lack the immunosuppressive properties associated with an intact rapamycin nucleus. The
15 rapamycin dialdehydes of this invention include compounds which may be chemically or biologically modified as derivatives of the rapamycin dialdehyde nucleus, while still retaining neurotrophic properties. Accordingly, the term "a rapamycin dialdehyde" includes esters, ethers, oximes, hydrazones, and hydroxylamines of rapamycin, as well as rapamycin dialdehydes in which functional groups on the rapamycin
20 dialdehyde nucleus have been modified, for example through reduction or oxidation. The term "a rapamycin dialdehyde" also includes pharmaceutically acceptable salts of rapamycin dialdehydes, which are capable of forming such salts, either by virtue of containing an acidic or basic moiety.



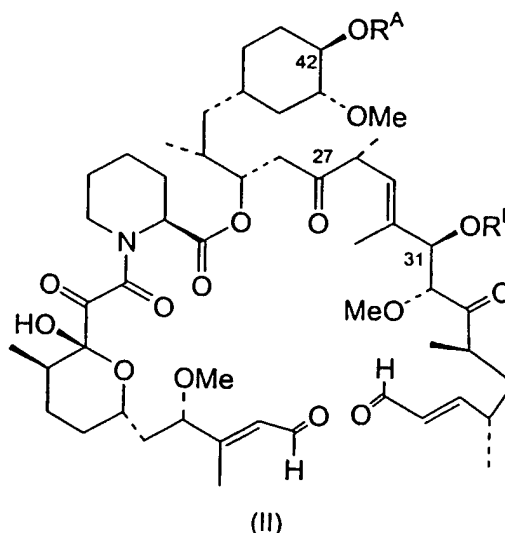
RAPAMYCIN DIALDEHYDE

It is preferred that the esters and ethers of rapamycin dialdehyde are of the hydroxyl groups at the 42- and/or 31-positions of the rapamycin dialdehyde nucleus, esters and ethers of a hydroxyl group at the 27-position (following chemical reduction of the 27-ketone), and that the oximes, hydrazones, and hydroxylamines are of a ketone at the 42-position (following oxidation of the 42-hydroxyl group) and of 27-ketone of the rapamycin dialdehyde nucleus.

Preferred 42- and/or 31-esters and ethers of rapamycin, which serve as starting materials for the corresponding rapamycin dialdehydes, are disclosed in the following patents, which are all hereby incorporated by reference: alkyl esters (U.S. Patent 4,316,885); aminoalkyl esters (U.S. Patent 4,650,803); fluorinated esters (U.S. Patent 5,100,883); amide esters (U.S. Patent 5,118,677); carbamate esters (U.S. Patent 5,118,678); silyl ethers (U.S. Patent 5,120,842); aminoesters (U.S. Patent 5,130,307); acetals (U.S. Patent 5,51,413); aminodiester (U.S. Patent 5,162,333); sulfonate and sulfate esters (U.S. Patent 5,177,203); esters (U.S. Patent 5,221,670); alkoxyesters (U.S. Patent 5,233,036); O-aryl, -alkyl, -alkenyl, and -alkynyl ethers (U.S. Patent 5,258,389); carbonate esters (U.S. Patent 5,260,300); arylcarbonyl and alkoxy carbonyl carbamates (U.S. Patent 5,262,423); carbamates (U.S. Patent 5,302,564); hydroxyesters (U.S. Patent 5,362,718); hindered esters (U.S. Patent 5,385,908); heterocyclic esters (U.S. Patent 5,385,909); gem-disubstituted esters

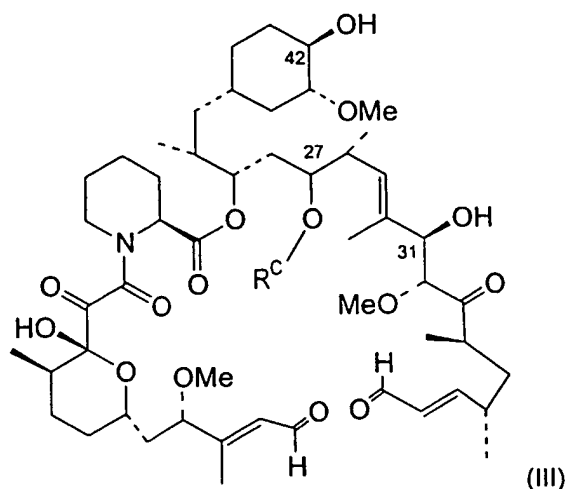
(U.S. Patent 5,385,910); amino alkanolic esters (U.S. Patent 5,389,639); phosphorylcarbamate esters (U.S. Patent 5,391,730); carbamate esters (U.S. Patent 5,411,967); carbamate esters (U.S. Patent 5,434,260); amidino carbamate esters (U.S. Patent 5,463,048); carbamate esters (U.S. Patent 5,480,988); carbamate esters (U.S. Patent 5,480,989); carbamate esters (U.S. Patent 5,489,680); hindered N-oxide esters (U.S. Patent 5,491,231); biotin esters (U.S. Patent 5,504,091); O-alkyl ethers (U.S. Patent 5,665,772); and PEG esters of rapamycin (U.S. Patent 5,780,462). The preparation of these esters and ethers are disclosed in the patents listed above. The preparation of the corresponding esters and ethers of rapamycin dialdehyde can be accomplished using the methodology described in these patents, starting with rapamycin, followed by the ring opening reactions described herein. An improved synthesis of 42-esters and ethers of rapamycin is disclosed in US Patent 6,277,539, which is hereby incorporated by reference.

Accordingly examples of rapamycin dialdehydes include rapamycin dialdehyde and 42- and/or 31- ester and ether compounds of formula (II):



wherein R^A and R^B are each selected from hydrogen and ester or ether forming groups as disclosed in any one of the abovementioned US/EP patents (e.g acyl groups and optionally substituted alkyl groups).

Preferred 27-esters and ethers of rapamycin, which serve as starting materials for the corresponding rapamycin dialdehydes, are disclosed in U.S. Patent 5,256,790, which is hereby incorporated by reference. The preparation of these esters and ethers are disclosed in the patents listed above. The preparation of the corresponding esters and ethers of rapamycin dialdehyde can be accomplished using the methodology described in these patents, starting with rapamycin, followed by the ring opening reactions described herein. Accordingly examples of rapamycin dialdehyde 27- ester and ether compounds are those of formula (III):



10

wherein R^C is hydrogen or an ester or ether forming groups as disclosed in US Patent 5,256,790.

Preferred oximes, hydrazones, and hydroxylamines of rapamycin are disclosed in U.S. Patents 5,373,014, 5,378,836, 5,023,263, 5,023,264, and 5,563,145, which are hereby incorporated by reference. The preparation of these oximes, hydrazones, and hydroxylamines, which serve as starting materials for the corresponding rapamycin dialdehydes, are disclosed in the above listed patents. The preparation of the corresponding oximes, hydrazones, and hydroxylamines of rapamycin dialdehyde can be accomplished using the methodology described in these patents, starting with rapamycin, followed by the ring opening reactions described herein.

20

Particularly preferred rapamycin dialdehydes include rapamycin dialdehyde, rapamycin dialdehyde 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid [see U.S. Patents 5,362,718 and 6,277,539 for the preparation of rapamycin
5 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid], and 42-O-(2-hydroxy)ethyl rapamycin dialdehyde [see U.S. Patent 5,665,772 for the preparation of 42-O-(2-hydroxy)ethyl rapamycin].

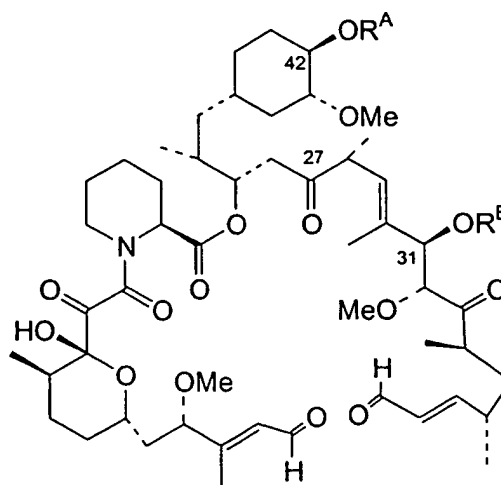
When applicable, pharmaceutically acceptable salts can be formed from
10 organic and inorganic acids, for example, acetic, propionic, lactic, citric, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, phthalic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, naphthalenesulfonic, benzenesulfonic, toluenesulfonic, camphorsulfonic, and similarly known acceptable
15 aids when the rapamycin contains a suitable basic moiety. Salts may also be formed from organic and inorganic bases, such as alkali metal salts (for example, sodium, lithium, or potassium) alkaline earth metal salts, ammonium salts, alkylammonium salts containing 1-6 carbon atoms or dialkylammonium salts containing 1-6 carbon
20 atoms in each alkyl group, and trialkylammonium salts containing 1-6 carbon atoms in each alkyl group, when the rapamycin contains a suitable acidic moiety.

As used in accordance with this invention, the term "providing," with respect to providing a compound or substance covered by this invention, means either directly administering such a compound or substance, or administering a prodrug, derivative,
25 or analog which will form the effective amount of the compound or substance within the body.

The compounds of this invention can be made from a rapamycin using commercially available starting materials or from starting materials that can be prepared according to literature procedures. The desired rapamycin dialdehyde can
30 be prepared by the treatment of the rapamycin with an oxidizing agent such as OsO₄/sodium periodate or ozone by monitoring the progress of the reaction, until the desired product has formed.

Accordingly this invention provides a process for preparing a rapamycin
35 dialdehyde as defined herein which comprises one of the following:

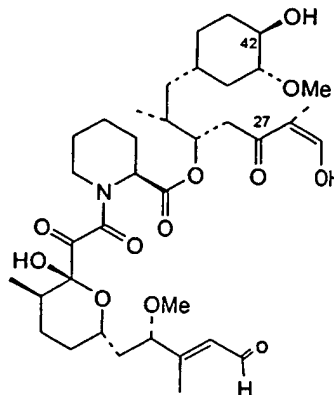
- a) ozonolysing a rapamycin (e.g. rapamycin, rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid, or 42-O-(2-hydroxy)ethylrapamycin) in a non-
to produce a rapamycin dialdehyde (e.g rapamycin dialdehyde, rapamycin dialdehyde
42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid or 42-O-(2-
5 hydroxy)ethyl rapamycin dialdehyde);
or
b) acylating rapamycin dialdehyde, optionally protected at reactive substituent
group(s), with an acylating agent (e.g. an acylating agent containing the group R^A or
 R^B as defined above) to give a rapamycin dialdehyde, e.g. a 42-, 31- or 42,31- ester
10 derivative thereof of formula (II):



(II)

- wherein R^A and R^B are each selected ester forming groups (e.g. acyl radicals) or one
of R^A and R^B is hydrogen), and removing any protecting group;
or
15 c) etherifying rapamycin dialdehyde optionally protected at reactive substituent
group(s) with an etherifying agent (e.g. an etherifying agent containing the group R^A or
 R^B as defined above) to give a rapamycin dialdehyde (e.g. a 42-, 31- or 42,31- ether
derivative thereof of formula (II) as shown above wherein R^A and R^B are each selected
ether forming groups or one of R^A and R^B is hydrogen), and removing any protecting
20 group.

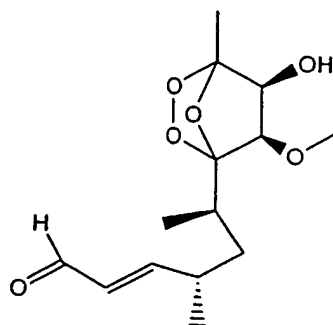
In addition to the production of the rapamycin dialdehyde, corresponding rapamycin 29-enols are also produced during the ozonolysis oxidation. The structure of rapamycin 29-enol is provided below.



RAPAMYCIN 29-ENOL

5

Additionally, during the ozonolysis of the rapamycin, (2E,4S,6R)-6-[(5S,6R)-5-hydroxy-6-methoxy-4-methyl-2,3,7-trioxabicyclo[2.2.1]hept-1-yl]-4-methylhept-2-enal, having the structure shown below was produced (Example 3).



10

The neuroregeneration activity for the compounds of this invention was confirmed in a standard pharmacological test procedure by evaluating rapamycin dialdehyde (Example 1) and rapamycin 29-enol (Example 2), as representative compounds of this invention, against SH-SY5Y cells *in vitro* [Gold et al., Exp Neurol 147: 269-278, 1997]. Briefly, SH-SY5Y cells were placed in 6-well plate treated with aphidicolin for 5 days followed by the test compounds using 6-well plates. For

controls, wells were untreated, or treated with nerve growth factor (NGF) alone. Test wells were treated with NGF plus Example 1 and Example 2, or rapamycin. Cells were photographed at 168 hours. Analysis of neuritic lengths was performed on photographic prints using a Houston Instrument HI-PAD digitizing tablet connected to an IBM XT computer with appropriate software (Bioquant IV, R&M Biometrics, Nashville, TN). Mean values for axonal areas were compared using by ANOVA (STATVIEW, Abacus Concepts, Inc., Berkeley, CA). The following table summarizes the results that were obtained.

Table I. Mean Neurite Lengths after 168 h

	Neurite Length (μm)
Untreated Cell	109.5 ± 2.6^a (n=115)
NGF	$196.5 \pm 7.7^*$ (n=112)
Rapamycin + NGF	$261.4 \pm 13.1^{**}$ (n=119)
Example 1 + NGF	$229.8 \pm 9.5^{**}$ (n=141)
Example 2 + NGF	$239.7 \pm 10.0^{**}$ (n=155)

^a Values are mean \pm SEM (in μm). n, number of cells.

* $p < 0.05$ compare to Untreated Cell

** $p < 0.05$ compare to NGF and Untreated Cell

The results obtained in the standard pharmacological test procedure demonstrated that the compounds of this invention are useful as neurotrophic agents, and are particularly useful in promoting neuronal regeneration and functional recovery and to stimulate neurite outgrowth and thereby to treat various neuropathological states, including damage to peripheral nerves and the central nervous system caused by physical injury (e.g., spinal cord injury and trauma, sciatic or facial nerve lesion or injury), disease (e.g., diabetic neuropathy), cancer chemotherapy (e.g., by vinca alkaloids and doxorubicin), brain damage associated with stroke and ischemia associated with stroke, and neurological disorders including, but not limited to, various peripheral neuropathic and neurological disorders related to neurodegeneration including, but not limited to: trigeminal neuralgia, glossopharyngeal neuralgia, Bell's palsy, myasthenia gravis, muscular dystrophy, amyotrophic lateral sclerosis, progressive muscular atrophy, progressive bulbar inherited muscular atrophy,

- herniated, ruptured or prolapsed vertebral disk syndromes, cervical spondylosis, plexus disorders, thoracic outlet destruction syndromes, peripheral neuropathies such as those caused by lead, acrylamides, gamma-diketones (glue-sniffer's neuropathy), carbon disulfide, dapsone, ticks, porphyria, Gullain-Barre syndrome, dementia, Alzheimer's disease, Parkinson's disease, and Huntington's chorea.

Antineoplastic activity for the compounds of this invention was confirmed by evaluating the antineoplastic activity of representative compounds of this invention (Examples 1-5) against six tumor cell lines *in vitro*. Briefly, tumor cells from six cell lines were placed in wells of a 96 well microtiter plate. The following tumor cell lines were used: 3T3 (ovarian), 3T3/H2N (ovarian – resistant to cis-platin), A431 (vulva epidermoid origin), SW620 (colon), SKBR3 (breast), and MDA-435 (breast). The tumors cells were grown in the presence of serial dilutions of the compound to be evaluated for 48 hours, and cell growth determined using a colorimetric procedure (sulforhodamine B). The inhibition of growth was calculated compared to the number of cells at the time of test compound addition. Results are expressed as an IC₅₀ (μg/ml); and are provided in Table 2.

Table 2. Antineoplastic Activity (IC₅₀ in μg/ml)

Cell line	Example 1	Example 2	Example 3	Example 4	Example 5
3T3	0.72	>5	4.11	1.5	>5
3T3/H2N	0.68	>5	>5	0.72	4.96
A431	0.91	>5	3.01	2.64	>5
SKBR3	0.52	>5	2.05	0.36	2.69
MDA435	1.23	>5	4.13	1.1	>5
SW620	1.41	>5	>5	3.04	>5

The results of this standard pharmacological test procedure demonstrate that the compounds of this invention are useful as antineoplastic agents. In particular, the compounds of this invention are useful against solid tumors, including sarcomas and carcinomas; and more particularly against astrocytomas, prostate cancer, breast cancer, colon cancer, small cell lung cancer, and ovarian cancer; and adult T-cell leukemia/lymphoma.

The compounds of this invention are also useful in the treatment or inhibition of hyperproliferative vascular diseases such as restenosis

5 When used for restenosis, it is preferred that the compounds of this invention are used to treat restenosis that occurs following an angioplasty procedure. When used for this treating restenosis following an angioplasty, the compounds of this invention can be administered prior to the procedure, during the procedure, subsequent to the procedure, or any combination of the above.

10

 It is understood that the effective dosage of a rapamycin dialdehyde may vary depending upon the particular compound utilized, the mode of administration, the condition, and severity thereof, of the condition being treated, as well as the various physical factors related to the individual being treated. As used in accordance with
15 invention, satisfactory results may be obtained when the rapamycin dialdehyde is administered in a daily oral dosage of from about projected daily dosages of active compound would be 0.1 µg/kg - 100 mg/kg, preferably between 0.001 - 25 mg/kg, and more preferably between 0.01 - 5 mg/kg. The projected daily dosages are expected to vary with route of administration.

20

 When the rapamycin dialdehyde is used as part of a combination regimen, dosages of each of the components of the combination are administered during a desired treatment period. The components of the combination may administered at the same time; either as a unitary dosage form containing both components, or as
25 separate dosage units; the components of the combination can also be administered at different times during a treatment period, or one may be administered as a pretreatment for the other.

 Such doses may be administered in any manner useful in directing the active
30 compounds herein to the recipient's bloodstream, including orally, via implants, parenterally (including intravenous, intraperitoneal and subcutaneous injections), rectally, intranasally, vaginally, and transdermally. For the purposes of this disclosure, transdermal administrations are understood to include all administrations across the surface of the body and the inner linings of bodily passages including epithelial and

mucosal tissues. Such administrations may be carried out using the present compounds, or pharmaceutically acceptable salts thereof, in lotions, creams, foams, patches, suspensions, solutions, and suppositories (rectal and vaginal).

5 Oral formulations containing the active compounds of this invention may comprise any conventionally used oral forms, including tablets, capsules, buccal forms, troches, lozenges and oral liquids, suspensions or solutions. Capsules may contain mixtures of the active compound(s) with inert fillers and/or diluents such as the pharmaceutically acceptable starches (e.g. corn, potato or tapioca starch), sugars,
10 artificial sweetening agents, powdered celluloses, such as crystalline and microcrystalline celluloses, flours, gelatins, gums, etc. Useful tablet formulations may be made by conventional compression, wet granulation or dry granulation methods and utilize pharmaceutically acceptable diluents, binding agents, lubricants, disintegrants, surface modifying agents (including surfactants), suspending or
15 stabilizing agents, including, but not limited to, magnesium stearate, stearic acid, talc, sodium lauryl sulfate, microcrystalline cellulose, carboxymethylcellulose calcium, polyvinylpyrrolidone, gelatin, alginic acid, acacia gum, xanthan gum, sodium citrate, complex silicates, calcium carbonate, glycine, dextrin, sucrose, sorbitol, dicalcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, talc, dry
20 starches and powdered sugar. Preferred surface modifying agents include nonionic and anionic surface modifying agents. Representative examples of surface modifying agents include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminum silicate,
25 and triethanolamine. It is more preferred that poloxamer 188 is used as the surface modifying agent. Oral formulations herein may utilize standard delay or time release formulations to alter the absorption of the active compound(s). Preferred oral formulations of rapamycin is disclosed in U.S. Patents 5,559,121; 5,536,729; 5,989,591; and 5,985,325, which are hereby incorporated by reference.

30

In some cases it may be desirable to administer the compounds directly to the airways in the form of an aerosol.

The compounds of this invention may also be administered parenterally or intraperitoneally. Solutions or suspensions of these active compounds as a free base

or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparation contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils. Preferred parenteral formulations for administering rapamycin are disclosed in U.S. Patents 5,530,006; 5,516,770; and 5,616,588, which are hereby incorporated by reference.

Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting point, and glycerin. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used.

When the rapamycin dialdehyde is used to treat or inhibit a hyperproliferative vascular disorder, such as restenosis, it is preferable that the rapamycin dialdehyde is provided via a vascular stent or shunt which has been coated or impregnated with the rapamycin dialdehyde.

The following provides procedures for the preparation of representative compounds of this invention.

EXAMPLE 1 (method A)

Rapamycin dialdehyde

Rapamycin (5.0 g, 5.5 mmole) was dissolved in 100 mL dioxane and 35 mL water. Then 61 mg of OsO₄ was added and reaction was carried out at room temp. After stirred 30 min (solution became dark green). Sodium metaperiodate (2.34 g, 10.9 mmole) was added batch by batch in 30 min. The reaction was stirred overnight.

- The reaction was stopped by extracting reaction mixture by 200 mL CH₂Cl₂ and work-up to get 5.1 g of crude product (66% of total peak area for title compound, 12% unreacted rapamycin). The crude product was purified by preparative HPLC on a Prep Nova-pak HR C18 column (300 x 19 mm) using gradient method that held 65% A and 35% B for the first 5 min then changed from 65% A and 35% B to 10% A and 90 % B in 30 min. Buffer A is 90% water and 10 % acetonitrile. Buffer B is 10% water and 90% acetonitrile. The flow rate is 20 mL/min. The fraction at 20-21 min was collected and extracted with methylene chloride. The organic layer was dried with anhydrous sodium sulfate. The organic solvent was removed using a rotary evaporation system. The residual was dissolved in 3 mL methylene chloride and precipitated by adding 15 mL hexane. After filtration, the white solid was dried in the speed-vac overnight. If the product is not pure enough, it was repurified by 51% B and 49%A on same column. The peak at 13 min was collected, work up again as described above. A white solid was obtained.

15

Assignments for Compound I, DMSO-d₆, 400 MHz (¹³C: 100 MHz)

C#	$\delta^{13}\text{C}$ major	$\delta^{13}\text{C}$ minor	$\delta^1\text{H}$ major	$\delta^1\text{H}$ minor	^1H Correltn	HMBC
1	163.34		6.91		2,36	3,35,36,50
2	131.37		6.12		1,3	3,36
3	194.38		9.52		2	2
4	192.08	191.92	10.02		5	5
5	127.54	128.07	6.06	6.00	4	4,7,45
6	160.98	161.23				
7	81.78	81.87	3.78	3.76	8	5
8	39.41	39.08	1.62;1.62			
9	65.97		3.74			
10	32.81		1.76 (eq) 1.15 (ax)			
11	26.33		1.50			
12	34.68	34.49	2.00			
14	99.27	99.98				
14 (OH)			6.44	6.53		12,13,15
15	198.98	199.45				
16	166.06	166.89				
18	43.42	37.94	3.51 (eq) 3.10 (ax)	4.22 (eq) 2.56 (ax)		
19	24.30		1.63 (eq) 1.27 (ax)			
20	20.70		1.75			
21	26.87		2.14 (eq) 1.64 (ax)			
22	50.92	54.78	4.96	4.46		
23	169.47	168.77				

C#	$\delta^{13}\text{C}$ major	$\delta^{13}\text{C}$ minor	$\delta^1\text{H}$ major	$\delta^1\text{H}$ minor	^1H Correltn	HMBC
25	74.10		5.17		26,37	
26	39.95		2.72 2.67			
27	208.15	208.37				
28	45.51		3.45			
29	126.06		5.21		28,48(w)	
30	138.00					
31	75.95		4.01		31-OH, 32	
31 (OH)			5.26		31	30,32
32	86.04		3.78			
33	212.53					
34	40.05		2.80			
35	37.63		2.56;1.22			
36	33.85		2.55			
37	32.55		1.83			
38	38.08		1.16;1.02			
39	32.42		1.32			
40	35.24	35.20	1.94 (eq) 0.62 (ax)			
41	83.77		2.83			
42	73.06		3.19			
42 (OH)			4.53			
43	32.81		1.74 (eq) 1.22 (ax)			
44	30.92		1.59 (eq) 0.88 (ax)			
45	11.82		2.06			
46	15.61		0.69			
47	15.70		1.00			
48	11.38		1.70			
49	15.29		0.95			
50	19.76		1.06			
51	15.06		0.80			
52	55.93		3.11			
53	57.55		3.19			
54	56.57		3.30			

* The pseudo-molecular ions were observed with the $[\text{M}-\text{H}]^-$ ion at m/z 944 and $[\text{M}+\text{NH}_4]^+$ ion at m/z 963 by negative and positive electrospray modes, respectively.

5

EXAMPLE 1 (method B)

Rapamycin dialdehyde

Rapamycin (1.0 g, 1.09 mmol) was dissolved in 250 mL CH_2Cl_2 . The round bottom flask was put into a dry ice bath. The ozone gas was bubbling through the solution for 6 min (6 L/min, at output 7 of control knob setting). Then the reaction mixture was stirred 1 h. Methyl sulfide (100 μL) was added and the reaction was stirred

for another 1 h. Reaction was stopped by adding 200 mL water into reaction mixture. About 0.97 g of crude product was obtained after work-up (off-white solid, 44% of total peak area for title compound). LC/MS results prove that the major peak is the title compound.

5

EXAMPLES 2 and 3

Rapamycin 29-enol (Example 2) and

(2E,4S,6R)-6-[(5S,6R)-5-Hydroxy-6-methoxy-4-methyl-2,3,7-trioxabicyclo[2.2.1]hept-1-yl]-4-methylhept-2-enal (Example 3)

10

Rapamycin (1.3 g, 1.40 mmol) was treated same procedure as Example 1B except the ozone gas was bubbling for 50 min. About 1.1 g of crude product was obtained after work-up (off-white solid, 44% of total peak area for the dialdehyde of Example 2). Two major peaks (9.5 min and 14 min) were isolated by preparative HPLC. Example 2 (14 min) was obtained as white solid (50 mg) and Example 3 (9.5 min) was obtained as clear oil (23 mg).

15

Assignments for Example 2 in DMSO-d₆ at 30 °C, 400 MHz (¹³C: 100 MHz)

C#	$\delta^{13}\text{C}$ major	$\delta^{13}\text{C}$ minor	$\delta^1\text{H}$ major	$\delta^1\text{H}$ minor
4	192.24	192.07	10.02	10.01
5	128.29	127.57	6.07	5.99
6	161.10	161.46		
7	81.94	81.79	3.76	3.77
8	39.22	39.04	1.60; 1.60	1.74; 1.74
9	65.97	65.97	3.73	3.73
10	30.85	30.93*	1.27	1.27
11	227.23	27.23	1.45	1.50
12	34.82	34.66	2.09	2.01
14	99.34	99.04		
14(OH)			6.56	6.48
15	199.18	199.59		
16	166.12	166.85		
18	43.54	37.90	3.44 (eq) 3.13 (ax)	4.16 (eq) 2.60 (ax)
19	24.17	24.51	1.55 (eq) 1.34 (ax)	1.59 (eq) 1.21 (ax)
20				
21	26.49	26.32	2.12 (eq) 1.58 (ax)	2.08 (eq) 1.56 (ax)
22	51.00	55.93	4.92	4.44
23	169.61	168.82		
25	75.56	75.33	5.29	5.22

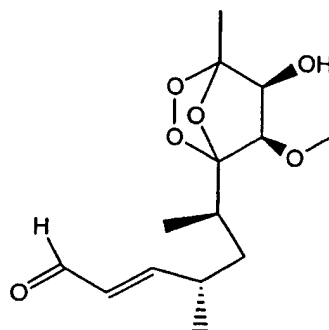
C#	$\delta^{13}\text{C}$ major	$\delta^{13}\text{C}$ minor	$\delta^1\text{H}$ major	$\delta^1\text{H}$ minor
26	35.94	36.74	2.87 2.66	2.76 2.76
27	196.03	196.03		
28	115.12	114.96		
29	157.38	157.16	7.83	7.78
29-OH			10.76	10.76
37	32.77	33.15	1.86	1.81
38	38.39	38.49	1.24 1.07	1.21 1.02
39	31.04	30.93*	1.33	1.30
40	35.49	34.49	1.96 (eq) 0.64 (ax)	1.94 (eq) 0.61 (ax)
41	83.87	83.87	2.83	2.81
42	75.56	75.33	3.18	3.12
42 (OH)			4.60	4.44
43	32.99	32.99	1.76 (eq) 1.17 (ax)	1.55 (eq) 1.34 (ax)
44	30.63	30.52	1.60 (eq) 0.88 (ax)	1.60 (eq) 0.88 (ax)
45	11.51	12.04	2.05	2.06
46	15.40	15.50	0.68	0.73
47	7.73	7.76	1.55	1.55
51	15.03	15.36	0.90	0.85
52	55.99	56.11	3.10	3.11
54	56.59	56.62	3.31	3.31

The pseudo-molecular ions for compound II were observed with the $[\text{M}-\text{H}]^-$ ion at m/z 706 and fragment ion 424.

5

For Example 3, ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ 9.5(1H, -CHO), 6.8 (1H, C=CH), 6.1 (1H, C=CH), 5.07 (1H, OH), 3.88, 3.76, 3.3 (3H, $-\text{OCH}_3$), 2.6, 2.2, 1.7, 1.5, 1.4, 1.10. ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ 194.7, 163.5, 131.6, 112.0, 110.4, 81.1, 73.9, 59.0, 35.6, 28.0, 20.3, 14.0. The pseudo-molecular ions for Example 3 were observed with the $[\text{M}-\text{H}]^-$ ion at m/z 301 and $[\text{M}+\text{NH}_4]^+$ ion at m/z 320 by negative and positive electrospray modes, respectively.

10



Chemical structure for Example 3, (2E,4S,6R)-6-[(5S,6R)-5-hydroxy-6-methoxy-4-methyl-2,3,7-trioxabicyclo[2.2.1]hept-1-yl]-4-methylhept-2-enal

5

Examples 4 and 5

42-O-(2-Hydroxy)ethyl rapamycin dialdehyde (Example 4) and

42-O-(2-Hydroxy)ethyl rapamycin 29-enol (Example 5)

42-O-(2-hydroxy)ethyl rapamycin (10 mg, 0.01 mmole) was dissolved in 10 mL
 10 CH₂Cl₂. The round bottom flask was put into a dry ice bath. The ozone gas was
 bubbling through the solution for 1 min (2 L/min, at output 7 of control knob setting).
 Then the reaction mixture was stirred 1 h. Methyl sulfide (5 μ l) was added and the
 reaction was stirred for another 0.5 h. About 9 mg of crude product was obtained after
 work-up. After Preparative HPLC, 0.5 mg of Example 4 was obtained and 0.24 mg of
 15 Example 5 was obtained.

The negative ion for Example 4 mode showed the [M-H]⁻ ion at m/z 988.6. By
 using rapamycin and 42-O-(2-hydroxy)ethyl rapamycin as internal standards, the
 exact mass measurement for the unknown ion was 988.5612 Da with elemental
 composition of C₅₃H₈₂NO₁₆ (theoretical mass = 988.5634, Δ = 2.2 mDa). Example 4
 20 compound contained two more oxygen to compare with 42-O-(2-hydroxy)ethyl
 rapamycin. Molecular weight was further confirmed by the positive electrospray mode
 to show a strong [M+NH₄]⁺ ion at m/z 1007.6.

Example 5 showed a pseudo-molecular ion at m/z 769.4 ([M+NH₄]⁺). The
 measured exact mass of 769.4487 Da corresponds to the elemental composition of
 25 C₃₉H₆₅N₂O₁₃ (theoretical mass = 769.4487, Δ = 0.0 mDa).

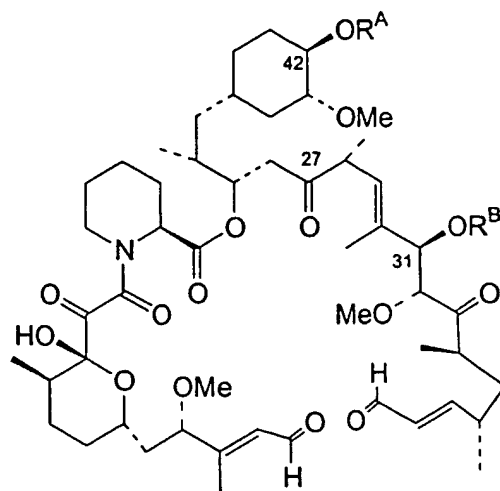
CLAIMS:

1. A compound which is a rapamycin dialdehyde or a pharmaceutically acceptable salt thereof.
- 5 2. The compound according to claim 1, wherein the rapamycin dialdehyde is rapamycin dialdehyde.
3. The compound according to claim 1, wherein the rapamycin dialdehyde is a
10 ester, ether, oxime, hydrazone, or hydroxylamine of rapamycin dialdehyde.
4. The compound according to claim 3, wherein the rapamycin is a 42-ester or 42-ether of rapamycin dialdehyde.
- 15 5. The compound according to claim 4, wherein the rapamycin is rapamycin dialdehyde 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid.
6. The compound according to claim 4, wherein the rapamycin is 42-O-(2-hydroxy)ethyl rapamycin dialdehyde.
- 20 7. A method of stimulating nerve cell growth in a mammal in need thereof, which comprises providing said mammal with an effective amount of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof.
- 25 8. A method of stimulating nerve regeneration in a mammal in need thereof, which comprises providing said mammal with an effective amount of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof.
- 30 9. A method of stimulating the growth of damaged peripheral nerves in a mammal in need thereof, which comprises providing said mammal with an effective amount of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof.

10. A method of treating or inhibiting a neurodegenerative disorder in a mammal in need thereof, which comprises providing said mammal with an effective amount of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof.
11. The method according to claim 10, wherein the neurodegenerative disorder is dementia, Parkinson's disease, Alzheimer's disease, or amyotrophic lateral sclerosis.
12. A method of treating peripheral nerve damage in a mammal in need thereof, which comprises administering to said mammal an effective amount of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof.
13. The method according to claim 12 wherein the peripheral nerve damage is caused by a physical injury or trauma.
14. The method according to claim 13, wherein the peripheral nerve damage is caused by spinal cord injury and trauma, sciatic or facial nerve lesion or injury.
15. A method of treating a solid tumor in a mammal in need thereof, which comprises providing to said mammal an effective amount of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof.
16. A method according to claim 15, wherein the solid tumor is selected from the group consisting of astrocytomas, prostate cancer, breast cancer, colon cancer, small cell lung cancer, and ovarian cancer; and adult T-cell leukemia/lymphoma.
17. A method of treating or inhibiting a hyperproliferative vascular disorder which comprises providing to said mammal an effective amount of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof.
18. A method according to claim 17, wherein the hyperproliferative vascular disorder is restenosis.

19. A method according to claim 18, wherein the rapamycin dialdehyde is administered via a vascular stent or shunt.
- 5 20. Use of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof in the preparation of medicament for stimulating nerve cell growth in a mammal.
- 10 21. Use of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof in the preparation of medicament for stimulating nerve regeneration in a mammal.
- 15 22. Use of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof in the preparation of medicament for stimulating the growth of damaged peripheral nerves in a mammal.
- 20 23. Use of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof in the preparation of medicament for treating or inhibiting a neurodegenerative disorder in a mammal.
24. Use according to claim 23, wherein the neurodegenerative disorder is dementia, Parkinson's disease, Alzheimer's disease, or amyotrophic lateral sclerosis.
- 25 25. Use of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof in the preparation of medicament for treating peripheral nerve damage in a mammal.
- 30 26. Use according to claim 25 wherein the peripheral nerve damage is caused by a physical injury or trauma.
27. Use according to claim 26, wherein the peripheral nerve damage is caused by spinal cord injury and trauma, sciatic or facial nerve lesion or injury.

28. Use of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof in the preparation of medicament for treating a solid tumor in a mammal.
- 5 29. Use according to claim 28, wherein the solid tumor is selected from the group consisting of astrocytomas, prostate cancer, breast cancer, colon cancer, small cell lung cancer, and ovarian cancer; and adult T-cell leukemia/lymphoma.
30. Use of a rapamycin dialdehyde as claimed in any one of claims 1 to 6 or a
10 pharmaceutically acceptable salt thereof in the preparation of medicament for treating or inhibiting a hyperproliferative vascular disorder in a mammal.
31. Use according to claim 30, wherein the hyperproliferative vascular disorder is restenosis.
- 15 32. Use according to claim 31, wherein the rapamycin dialdehyde is administered via a vascular stent or shunt.
33. A pharmaceutical composition which comprises a rapamycin dialdehyde as
20 claimed in any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.
34. A process for preparing a rapamycin dialdehyde as defined in any one of claims 1 to 6 which comprises one of the following:
- 25 a) ozonolysing a rapamycin (e.g. rapamycin, rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid, or 42-O-(2-hydroxy)ethylrapamycin) in a non- to produce a rapamycin dialdehyde (e.g rapamycin dialdehyde, rapamycin dialdehyde 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid or 42-O-(2-hydroxy)ethyl rapamycin dialdehyde);
- 30 or
- b) acylating rapamycin dialdehyde, optionally protected at reactive substituent group(s), with an acylating agent (e.g. an acylating agent containing the acyl group R^A or R^B as defined above) to give a rapamycin dialdehyde, e.g. a 42-, 31- or 42,31-ester derivative thereof of formula (II):



(II)

wherein R^A and R^B are each selected ester forming groups or one of R^A and R^B is hydrogen), and removing any protecting group;

5 or

c) etherifying rapamycin dialdehyde optionally protected at reactive substituent group(s) with an etherifying agent (e.g. an etherifying agent containing an ether forming group R^A or R^B as defined above) to give a rapamycin dialdehyde (e.g. a 42-,

31- or 42,31- ether derivative thereof of formula (II) as shown above wherein R^A and R^B are each selected ether forming groups or one of R^A and R^B is hydrogen), and
 10 removing any protecting group.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 02/26717

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D405/06 C07D493/08 A61K31/445 A61K31/335

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 09970 A (AMERICAN OME PRODUCTS CORPORATION) 12 March 1998 (1998-03-12) page 13 -page 22; claims 1-11 ---	1-34
Y	WO 96 17845 A (AMERICAN OME PRODUCTS CORPORATION) 13 June 1996 (1996-06-13) page 20 -page 29; claims 1-24 ---	1-34
A	WO 96 17816 A (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 13 June 1996 (1996-06-13) page 18 -page 23; claims 1-31 ---	1-6, 34
Y	WO 95 28406 A (AMERICAN OME PRODUCTS CORPORATION) 26 October 1995 (1995-10-26) page 20 -page 31; claims 1-25 & US 5 362 718 A 8 November 1994 (1994-11-08) --- -/--	1-34



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
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- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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& document member of the same patent family

Date of the actual completion of the international search

17 December 2002

Date of mailing of the international search report

30/12/2002

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Kyriakakou, G

INT' NATIONAL SEARCH REPORT

In International Application No

PCT/US 02/26717

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GB 2 247 456 A (FUJISAWA PHARMACEUTICAL CO., LTD) 4 March 1992 (1992-03-04) page 16 -page 18; claims 1-8	1-34
A	EP 0 378 318 A (MERCK & CO. INC.) 18 July 1990 (1990-07-18) the whole document	1-6, 34
Y	JOSEPH P. STEINER ET AL.: "Neurotrophic actions of nonimmunosuppressive analogues of immunosuppressive drugs FK506, rapamycin and cyclosporin A" NATURE MEDICINE., vol. 3, no. 4, April 1997 (1997-04), pages 421-428, XP002125244 NATURE PUBLISHING, CO., US ISSN: 1078-8956 the whole document	1-34
Y	DANIEL A. DICKMAN ET AL.: "Antifungal Rapamycin Analogues with reduced Immunosuppressive Activity" BIOORGANIC & MEDICINAL CHEMISTRY., vol. 10, 2000, pages 1405-1408, XP004222117 ELSEVIER SCIENCE LTD., GB ISSN: 0968-0896 the whole document	1-34
Y	RICHARD SEDRANI ET AL.: "Cleavage of the cyclohexyl-subunit of Rapamycin results in loss of immunosuppressive Activity" BIOORGANIC & MEDICINAL CHEMISTRY., vol. 9, 1999, pages 459-462, XP004157247 ELSEVIER SCIENCE LTD., GB ISSN: 0968-0896 the whole document	1-34
Y	DENOIS A. HOLT ET AL.: "Structure Activity studies of Nonmacroaclic Rapamycin Derivatives" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 13, no. 10, 1993, pages 1977-1980, XP000852549 OXFORD, GB ISSN: 0960-894X the whole document	1-34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 02/26717

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 7-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

In International Application No
PCT/US 02/26717

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9809970	A	12-03-1998	AU 4176897 A CN 1235608 A EP 0927182 A2 JP 2001500126 T WO 9809970 A2	26-03-1998 17-11-1999 07-07-1999 09-01-2001 12-03-1998
WO 9617845	A	13-06-1996	US 5563145 A AT 202357 T AU 714120 B2 AU 4417696 A BR 9509875 A CA 2206965 A1 CN 1174554 A ,B CZ 9701720 A3 DE 69521438 D1 DE 69521438 T2 DK 796260 T3 EP 0796260 A1 ES 2157353 T3 GR 3036209 T3 HK 1002361 A1 HU 77141 A2 JP 10510270 T NZ 298639 A PT 796260 T WO 9617845 A1 US 5677295 A	08-10-1996 15-07-2001 16-12-1999 26-06-1996 30-09-1997 13-06-1996 25-02-1998 14-01-1998 26-07-2001 16-05-2002 03-09-2001 24-09-1997 16-08-2001 31-10-2001 28-09-2001 02-03-1998 06-10-1998 29-11-1999 28-09-2001 13-06-1996 14-10-1997
WO 9617816	A	13-06-1996	US 5621108 A CA 2208131 A1 EP 0796236 A1 WO 9617816 A1	15-04-1997 13-06-1996 24-09-1997 13-06-1996
WO 9528406	A	26-10-1995	US 5362718 A AU 679854 B2 AU 2247495 A BR 9507323 A CA 2187024 A1 CN 1149296 A ,B CZ 9603052 A3 EP 0763039 A1 HU 77410 A2 IL 113179 A JP 9512018 T NZ 283988 A PL 316948 A1 RU 2134267 C1 SK 133096 A3 WO 9528406 A1 ZA 9503090 A	08-11-1994 10-07-1997 10-11-1995 30-09-1997 26-10-1995 07-05-1997 13-08-1997 19-03-1997 28-04-1998 15-06-1998 02-12-1997 28-07-1998 17-02-1997 10-08-1999 09-04-1997 26-10-1995 14-10-1996
GB 2247456	A	04-03-1992	NONE	
EP 378318	A	18-07-1990	CA 2007490 A1 EP 0378318 A1 JP 2233643 A US 5446158 A	11-07-1990 18-07-1990 17-09-1990 29-08-1995